

International
Research Journal of
**FOOD AND
NUTRITION**



Volume 02 | Issue 01 | 2020



SciRange
PUBLICATIONS

www.scirange.com

The Effect of Milk Fat Replacement by Some Edible Oils on Chemical Composition, Antioxidant Activity and Oxidative Stability of Spreadable Processed Cheese Analogues

¹I.H.I. Abdel-Ghany, ¹Sally S. Sakr, ²Mahassen M.M. Sleem and ³Hamdy A. Shaaban

¹Department of Dairy Science, Faculty of Agriculture, Cairo University, Giza, Egypt

²Department of Dairy, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt

³Department of Chemistry of Flavour and Aroma, NRC, Dokki, Giza, Egypt

ARTICLE INFORMATION

Received: January 28, 2020

Accepted: February 20, 2020

Corresponding Author:

Hamdy A. Shaaban,
Department of Chemistry of Flavour
and Aroma, NRC, Dokki, Giza, Egypt

ABSTRACT

This study was conducted to examine the effect of replacing milk fat by some edible oils (butter oil, rice bran oil and palm oil) on chemical composition, antioxidant activity and oxidative stability of full-fat, half fat and low-fat spreadable processed cheese analogues. In general, the chemical composition (total solids %, fat %, fat/dry matter, total protein % and ash %) was satisfied to the legal Egyptian standards (ES: 1132-2/2005) with no effect of the type of edible oil used or storage temperature (5 or 25°C) after three months. The pH value of fresh samples containing Palm Oil (PO) or Rice Bran Oil (RBO) were higher than that containing Butter Oil (BO). Generally, the pH values decreased in all spreadable processed cheese analogue samples at the end of storage period at both storage temperature used under this study (5 or 25°C). Regarding the antioxidant activity it was observed that the more fat percent in spreadable processed cheese analogue increased the more antioxidant effect of its methanol extract increased. The replacement of milk fat by RBO led to a higher radical scavenging inhibition and reducing power effect than samples containing PO or BO. All spreadable processed cheese analogues produced under this study showed slight changes in dienes and thiobarbituric acid (TBA) values which indicate their stability towards oxidation after three months of storage.

Key words: Processed cheese analogue, rice bran oil, palm oil, butter oil, antioxidant, oxidative stability

INTRODUCTION

Processed Cheese is an important part of the cheese market¹. In Egypt the production of processed cheese products are in increase and have received much attention². Normally, the plan of handled processed cheese should be possible by mixing diverse cheese types with various level of development, enhancing into smooth homogeneous mix with the guide of heat, mechanical shear and emulsifying salts^{3,4}. Recently, because of both economic reasons and the increase of consumer's awareness on the impact of food on health the use of partially or fully replacement of natural cheeses with several dairy or non-dairy ingredients. Solowiej⁵ have been used to produce processed cheese-like (cheese analogs) products^{6,7}. Regarding the water face, either rennet or acid

caseins or their combination are reported to be good sources of protein in production of processed cheese imitations⁵. That because of their functional characteristics to giving food products a suitable structure, consistency, fat emulsifying properties, whipping ability and bland flavor⁸.

In spite of, the decrease in fat substance of cheese has been prescribed to get useful products low in cholesterol and soaked unsaturated fats the lower fat or nonfat common cheese is related with numerous useful and organoleptic disadvantages⁹⁻¹¹. When fat is diminished an expansion in protein arrange and an abatement in grease gave by fat happens which brings about a firm, rubbery surface and poor melting characteristics^{12,13}. These defects can be enhanced by the addition of water, protein, or other additives such as gums and stabilizers^{1,14}. Adding fat replacers that partially or fully replace fat and simulate the properties of fat also can help to improve the acceptability of low fat processed cheese analogs^{15,16}. Another methodology is substituting of milk fat totally or partially by vegetable fats⁴. Lobato-calleros and Vernon-carter⁹ reported that the substitution of milk fat by different vegetable fats directly affect the characteristics of the resultant processed cheese analogous. The morphology, size and distribution of the fat droplets into the protein network were all influenced by the type of fat used which indicate that the type of fat affects the properties of the final^{4,17}.

In recent years, uses of Rice Bran Oil (RBO) in human nutrition are in increase. RBO has been used for centuries in Asian countries and its popularity is in increase in the United States, India and Thailand due to its health benefits¹⁸. RBO is balanced in saturated, monounsaturated and polyunsaturated fatty acids and meets the American Heart Association (AHA) recommendations¹⁹. It is also, rich in nutraceutical compounds such as tocopherols, tocotrienols and gamma oryzanol which they have been linked with health improvement by lowering serum cholesterol, having antioxidant functionality in humans²⁰⁻²². Numerous scientists got intrigued to contemplate the impact of milk fat substitution by RBO in cheese products. Tuntragul *et al.*²³ found that substitution of milk fat by RBO in without fat mozzarella cheese improved both useful and healthy attributes of produced cheese.

Thus, this study was planned to investigate the effect of three different types of edible fat (butter oil, palm oil and rice bran oil) on the chemical composition, antioxidant activity and stability of full, half and low fat spreadable processed cheese analogues.

MATERIALS AND METHODS

Raw materials: To manufacture full fat, half fat and low fat spreadable processed cheese analogues the following

ingredients were used: fresh skimmed buffalo's milk obtained from the herd of the Faculty of Agriculture, Cairo University, Giza, Egypt was used to produce acid and rennet caseins. S₉s special obtained from JOHABK Ladenburg, Germany was used as an emulsifying salt. Single quality fluid calf rennet was gotten from Dairy unit, Dairy science Department, Faculty of Agriculture, Cairo University and it was added to the milk at pace of 1.5 mL kg⁻¹ milk. Freeze-dried lactic culture for Direct Vat Set (YC-X11, thermophilic yoghurt culture Yo-Flex®) was obtained from Misr Food Additives Company, Giza, Egypt and it was used as a starter culture. Buffalo's butter oil was obtained after churning process in our lab. A commercial fine grade sodium chloride salt was obtained from El-Nasr saline's Co., Alexandria, Egypt. Palm oil was from Arma Company for Food Industries. RBO (free of cholesterol and contains 200 mg/100 g oil of Oryzanol) produced in Thailand was purchased from Egyptian local market.

Manufacture of spreadable processed cheese analogues

Production of protein source: Milk was pasteurized (72°C/15 sec), then at cooled 40°C before the addition of starter culture. Twenty minute later rennet was added until final coagulation and the curd occurred (42% TS, 2% fat and 4.76% F/DM). The second one (Curd 2) was made up by rennet coagulation only (29% TS, 1% fat and 3.45% F/DM). These curds were kept under freezing conditions until used.

Processing of the spreadable processed cheese analogues:

The full fat, half fat and low fat spreadable processed cheese analogues were prepared under the guide lines recommended by the Egyptian Organization for Standardization and quality. They were formulated under the regulations appeared in the Egyptian Standards (ES: 1132-2/2005) for processed cheese with vegetable oils and fats parts 1 and 2 as follows: 59% moisture, 41% TS, 18.45% fat and 45 F/DM for full fat, 69% moisture, 31% TS, 7.75% fat and 25 F/DM for half fat and 71% moisture, 29% TS, 4.35% fat and 15 F/DM for low fat.

Nine different blends of processed cheese analogues were manufactured in which they were different from each other in the source of fat (butter oil, palm oil or rice bran oil) or its percent (Full fat, half fat or low fat). The ingredients (g kg⁻¹ blend) used is illustrated in Table 1. Each blend was processed in a double jacketed pan using a direct steam for heating at 85-90°C for 5 min with continuous mixing at 1400 rpm. The hot semi-fluid processed cheese analogue was poured into plastic cups with screw and then cooled rapidly. Samples from each blend were divided into three groups. The first one was analyzed as fresh sample, the second was stored at 5°C for

Table 1: Blends composition of the spreadable processed cheese analogues

Spreadable processed cheese blend	Ingredient (g kg ⁻¹ blend)					
	Curd (1)	Curd (2)	Stabilizer salt (S ₉₉)	Salt	Fat	Water
Full fat	250	305.5	30	10	176.5	228.0
Half fat	250	330.4	30	10	69.2	310.4
Low fat	266	357.0	30	10	34.6	302.4

three months and the third was kept at 25 °C for three months as storage conditions before they were analyzed.

Analytical methods

Fatty acids profile analysis: For fatty acids relative distribution analysis of different fats the fatty acids methyl esters were produced according to AOAC²⁴ and the Gas liquid chromatography was used for determination on a Hewlett Packard Model 6890 chromatograph. Briefly, Separation was done on an INNO wax (polyethylene glycol) Model No. 19095 N-123, 240 °C maximum, capillary column 30.0 m × 530 µm × 1.0 µm, nominal flow 15 mL min⁻¹ with average velocity 148.78 cm sec⁻¹ and pressure 8.2 psi temperature programming: Initial temperature 100 to 240 °C final with 10 °C min⁻¹ for each minute and then holds at 240 °C for 10 min. Injection temperature 280 °C, back inlet, with split ratio 8:1, split flow 120 mL min⁻¹, gas saver 20 mL min⁻¹. Carrier gas was nitrogen with flow rate 15 mL min⁻¹. Flame ionization detector temperature 280 °C, hydrogen flow rate 30 mL min⁻¹ and air flow rate 300 mL min⁻¹.

Chemical composition and pH: Moisture and ash contents were determined as described by AOAC²⁵. Total nitrogen and fat contents were determined as mentioned by Ling²⁶. The pH values were measured by using pH meter: PTI-15, Aque chemical Co., England.

Antioxidant activity: The methanol extract from different spreadable processed cheeses samples was prepared by adding one gram of cheese sample to 10 mL methanol 98%, then blended well using carburetor magnetic and left for 24 h at 25 °C. The obtained extract was nominated using filter paper (whatman No. 1). The residue was re-extracted (3-4 times) with methanol. The filtrate left at room temperature even gets a sticky substance and kept in the dark at 4 °C until use²⁷. Then, the antioxidant activity properties of fresh spreadable processed cheese analogue samples were determined using two different methods as follow:

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity: Antioxidant capacity of fresh cheese analogue samples extracts was assessed using DPPH free radical. The

DPPH radical scavenging activity of oil extracts from cheese samples was quantified according to the method reported by Suwanna *et al.*²⁸ with minor modifications²⁹. Five different concentrations ranging between 0.1 and 1.0 mg mL⁻¹ of each extract (0.5 mL) was mixed with 2.5 mL of methanolic DPPH radical (0.1 mM). After leaving for 20 min in the dark at room temperature, the absorbance was recorded at wave length 517 nm using a UV visible spectrophotometer (UV 1601 PC, Spectrophotometer, Japan). The radical scavenging activity (%inhibition of DPPH) was calculated as a percentage of DPPH discoloration using the following equation:

$$\text{Inhibition of DPPH (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

(α-Tocopherol was used as the reference antioxidant).

Reducing power assay: The reducing power of cheese samples was carried out as described by Yildirim *et al.*²⁹ with minor modification compared to α-tocopherol (60 mg mM⁻¹). One milliliter of sample extracted was mixed with 1 mL of sodium phosphate buffer (0.2 M pH 6.6) and 1 mL of 1% potassium ferricyanide [K₃Fe(CN)₆]. The mixture was incubated at 50 °C for 30 min, afterwards, 1 mL of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 2000 g for 10 min. The upper layer (1 mL) was mixed with deionized water (1 mL) and 0.1% of ferric chloride (0.5 mL) and the absorbance was measured spectrophotometrically at wave length of 700 nm.

Oxidative stability: Edible oils used for milk fat replacement in the produced spreadable processed cheese analogues and cheese fat extracted from different cheese samples were analyzed for both peroxide (PV) and acid (AV) values as described in the AOAC²⁵. The thiobarbituric acid (TBA) value s determination was done according to method described by Keeny³⁰. Total Volatile Fatty Acids (TVFA) were determined as explained by Kosikowski³¹.

Determination of dienes content was also applied. Conjugated linoleic acids were estimated spectrophotometrically according to the method of AOAC³², as follows: about 1 g of processed cheese samples was weighed into 25 mL conical

flask, mixed with 2 g of sodium sulphate (anhydrous) and extracted three times with small amounts of petroleum ether. The combined extracts were transferred quantitatively into 25 mL volumetric flask and made up to volume with petroleum ether. One milliliter of the extract was diluted to 20 mL with petroleum ether. The absorbance of diluted solution was measured at wave length 233 nm using a spectrophotometer (spectrum uv-vis spectrophotometer, Taiwan). The absorbance was corrected for the calculation of diene content using the following equation:

$$a_2 = a_{233} - a_0$$

where, a_2 was the corrected absorptivity for conjugated dienes, a_{233} was the absorbance at 233 nm wavelength, a_0 was the correction factor which equals 0.07 (The percentage of conjugated dienes = $a_2 \times 0.84$).

RESULTS AND DISCUSSION

Characterization of edible oils: All edible oils used in the study were evaluated for their fatty acid composition, oxidative stability, acid and peroxide values. The fatty acids profile of different edible fats used in the study are presented in Table 2. The samples of the fat were also evaluated for their oxidative stability as well as acid and peroxide values AOAC²⁵ and the data are presented in Table 3.

Chemical composition and pH: The chemical composition of fresh manufactured spreadable processed cheese analogue samples and after three months of storage at 5 or 25°C was determined and presented in Table 4. The minimum percent of total solids content was detected for low fat cheese samples which ranged from 27.75 to 28.91% with no significant differences between all low fat spreadable processed cheese samples even they are different in the type of edible oil used. Among all cheese samples, the percent of total solids directly proportional with fat levels meeting the requirements of the quality standard. The fat content on dry matter basis of different spreadable processed cheese samples was satisfied to the legal Egyptian standards (ES: 1132-2/2005) and it was recorded to be round 43, 23 and 13 for full fat, half fat and low fat spreadable processed cheese samples respectively with no effect of the type of edible oil used or storage temperature after three months. The opposite trend was observed for total protein percent which is negatively proportional with the level of fat in all cheese samples represents no significant differences between cheese samples with the same level of fat. The use of different fat levels affected the values of ash

Table 2: Fatty acids profile and relative distribution (%) of edible oils used in the manufacture of spreadable processed cheese analogues

Fatty acid	Butter oil	Palm oil	Rice bran oil
Saturated	53.766	45.316	24.414
C4:0	1.699	-	-
C6:0	1.484	-	-
C8:0	0.828	-	-
C10:0	1.660	-	-
C11:0	-	-	-
C12:0	2.191	0.211	-
C14:0	0.411	0.997	0.424
C15:0	1.586	-	-
C16:0	29.701	39.219	19.680
C17:0	1.115	0.091	0.049
C18:0	12.280	4.248	2.137
C20:0	0.588	0.550	1.440
C22:0	0.223	-	0.273
C24:0	-	-	0.411
Monounsaturated	27.964	43.221	41.773
C14:1	0.433	-	-
C15:1	0.390	-	-
C16:1	1.744	0.219	0.207
C17:1	0.348	0.030	0.023
C18:1	25.049	42.972	41.543
Polyunsaturated	3.742	11.194	33.652
C18:2	2.978	10.898	32.456
C18:3	0.764	0.296	1.178
Non identified fatty acids	14.961	4.517	0.179

Table 3: Oxidative stability, acid value and peroxide value of edible oils used in the manufacture of spreadable processed cheese analogues

Type of edible fat	Oxidative stability at 110°C h ⁻¹	Acid value (mg KOH g ⁻¹ oil)	Peroxide value (meq O ₂ kg ⁻¹ oil)
Butter oil	7.80	0.41	1.44
Palm oil	2.40	0.24	2.72
Rice bran oil	5.75	1.02	2.04

content with no significant effect of edible oil type used. While it was affected by the storage temperature as shown in Table 4. The ash content in cheese samples stored for three months at 25°C were higher than those in fresh or stored at 5°C for three months.

The effect of milk fat replacement with different edible oils on the change of pH values in full-fat, half-fat and low-fat fresh spreadable processed cheese analogues and after three months of storage at 5 and 25°C was demonstrated at Fig. 1. The pH values (Fig. 1) of fresh processed cheese analogues containing vegetable oils (PO and RBO) were higher than that of containing BO. The fresh processed cheese analogue samples containing RBO had the highest amounts of pH than other samples and this may be due to the high acid value of ROB (1.02 mg KOH g⁻¹ oil) as presented in Table 1. The decrease of pH values for all samples at the end of storage period at 5 or 25°C was also observed. This is probably due to the hydrolysis of the emulsifying salts and their interaction

Table 4: Changes in chemical composition of produced spreadable processed cheese analogues under different storage temperature for three months

Age	Type	Treatment	TS (%)	Fat (%)	F/DM	TP (%)	Ash (%)
Fresh	Full	BO-F	40.11 ^a	17.43 ^{ab}	43.50 ^a	12.22 ^b	5.57 ^g
		RBO-F	40.46 ^a	17.53 ^{ab}	43.35 ^a	12.58 ^b	5.64 ^g
		PO-F	40.76 ^a	17.80 ^a	43.70 ^a	12.52 ^b	5.40 ^g
	Half	BO-H	30.45 ^b	7.07 ^c	23.22 ^b	13.23 ^b	5.61 ^g
		RBO-H	30.39 ^b	7.00 ^c	23.04 ^b	13.47 ^b	5.64 ^g
		PO-H	30.45 ^b	7.03 ^c	23.10 ^b	13.26 ^b	5.62 ^g
	Low	BO-L	28.46 ^{cd}	3.90 ^d	13.70 ^c	15.79 ^a	5.63 ^g
		RBO-L	28.91 ^d	3.97 ^d	13.73 ^c	15.86 ^a	5.90 ^{bcd}
		PO-L	28.57 ^d	3.97 ^d	13.90 ^c	16.05 ^a	6.04 ^{bcd}
3 months/5°C	Full	BO-F	40.01 ^a	17.33 ^{ab}	43.36 ^a	12.36 ^b	5.64 ^g
		RBO-F	40.33 ^a	17.40 ^{ab}	43.16 ^a	13.08 ^b	5.69 ^{efg}
		PO-F	40.66 ^a	17.42 ^{ab}	42.84 ^a	13.10 ^b	5.63 ^g
	Half	BO-H	30.37 ^b	6.90 ^c	22.72 ^b	13.53 ^b	5.76 ^{cdef}
		RBO-H	30.41 ^b	6.93 ^c	22.96 ^b	13.79 ^b	5.79 ^{cdef}
		PO-H	30.35 ^b	6.93 ^c	22.85 ^b	13.50 ^b	5.75 ^{cdef}
	Low	BO-L	28.26 ^d	3.80 ^d	13.37 ^c	15.86 ^a	5.80 ^{def}
		RBO-L	28.81 ^d	3.80 ^d	13.20 ^c	16.72 ^a	6.14 ^b
		PO-L	28.45 ^d	3.80 ^d	13.37 ^c	16.14 ^a	6.21 ^b
3 months/25°C	Full	BO-F	39.73 ^a	17.03 ^b	42.89 ^a	12.38 ^b	6.05 ^{bcd}
		RBO-F	40.17 ^a	17.10 ^b	42.58 ^a	13.01 ^b	6.15 ^b
		PO-F	40.45 ^a	17.43 ^{ab}	43.14 ^a	12.94 ^b	5.89 ^{bcd}
	Half	BO-H	30.07 ^b	6.50 ^c	21.63 ^b	13.72 ^b	6.11 ^{bc}
		RBO-H	29.97 ^{bc}	6.63 ^c	22.14 ^b	13.69 ^b	6.21 ^b
		PO-H	30.05 ^b	6.63 ^c	22.08 ^b	13.57 ^b	6.01 ^{bcd}
	Low	BO-L	28.14 ^d	3.47 ^d	12.39 ^c	16.22 ^a	6.12 ^g
		RBO-L	28.43 ^d	3.53 ^d	12.64 ^c	16.31 ^a	6.64 ^a
		PO-L	27.95 ^d	3.50 ^d	12.53 ^c	15.92 ^a	6.56 ^a
LSD			1.1116	0.5911	2.2847	1.6113	0.3392

BO-F: Full fat butter oil, BO-H: Half fat butter oil, BO-L: Low fat butter oil, RBO-F: Full rice bran oil, RBO-H: Half fat rice bran oil, RBO-L: Low fat rice bran oil, PO-F: Full fat palm oil, PO-H: Half fat palm oil, PO-L: Low fat palm oil, Different superscripts at the same column are significantly different ($p < 0.05$)

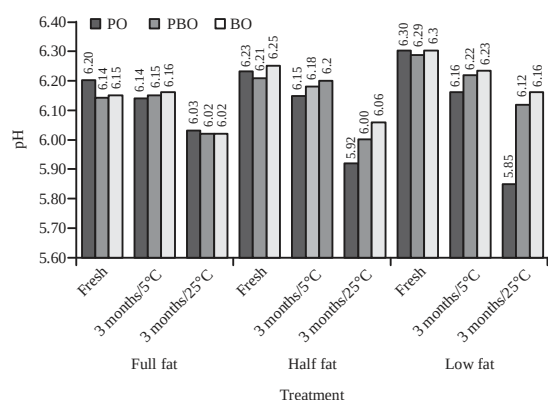


Fig. 1: Changes in pH value in different aged spreadable processed cheese analogue samples after three months under different storage temperature

with proteins as well as the free fatty acids degradation during storage^{3,33}. Replacement of milk fat by PO highly lowered the pH value which was the lowest for low-fat sample stored for three months at 25°C among all other samples. This finding can be supported by the results in Table 3 which showed that the oxidative stability at 110°C h⁻¹ for PO (2.40) was lower than those of RBO (5.75) and BO (7.80).

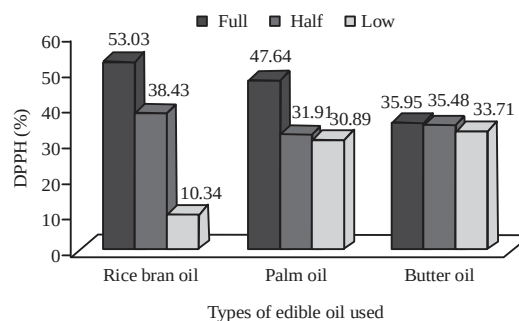


Fig. 2: Scavenging activity (DPPH %) of methanol extracts of fresh full, half and low spreadable processed cheese analogue samples as affected by different types of edible oils, BO: Butter Oil, RBO: Rice Bran Oil, PO-L: Low fat palm oil

Antioxidant activity of fresh spreadable processed cheese analogues:

The evaluation of antioxidant activity of fresh spreadable processed cheese analogues was studied and the degrees of radical scavenging activity (DPPH %), were presented in Fig. 2 and 3.

DPPH is a stable free radical that shows maximum absorbance at 517 nm. The amount of absorbance decreases as the radical

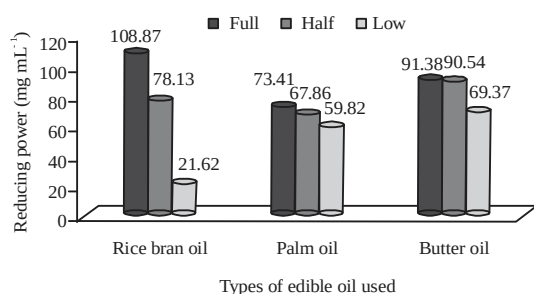


Fig. 3: Reducing power of methanol extracts prepared from fresh full-fat, half-fat and low-fat spreadable processed cheese analogue samples as affected by different types of edible oil

is scavenged by antioxidant substances as a result of neutralization of free radicals either by donates hydrogen or an electron by the tested sample³⁴. The inhibition percent in DPPH of methanol spreadable processed cheeses extracts as an antioxidant activity are largely related to the type of edible fats used (Fig. 2). Generally, the antioxidant activity was observed for full fat samples and relatively decreased by decreasing the fat level. Also, different full fat samples showed higher inhibition (DPPH %) than half or low fat samples. In the full fat spreadable processed cheese analogue samples the RBO had the higher radical scavenging inhibition (53.03%) among all other samples while it was the lowest for BO cheese sample (35.95%). Regarding the edible oils used, RBO (about 75.43% of unsaturated fatty acids) presented the highest antioxidant activity than PO (about 54.42% of unsaturated fatty acids) for all cheese types (Table 2 and Fig. 2, 3). These results can be supported by the finding of Valantina *et al.*³⁵ who reported that the antioxidant stability of RBO is greater than PO even under repeated thermal fluctuations. They explained that the more the unsaturated fatty acid in edible oil increased the more their antioxidant effect increased.

In the same Fig. 3 the reducing power activity for RBO spreadable processed cheese samples showed the higher levels (full, 108.87; half, 78.13 and low, 21.62) than those of PO which recorded to be 73.41, 67.86 and 59.82 in full, half and low fat samples respectively. It is well observed from Fig. 2 and 3 that the more fat percent in cheese increased the more antioxidant effect of its extract increased. Also, the antioxidant effect of RBO cheese samples is more than those of PO cheese samples. This finding is in parallel with those reported by Valantina *et al.*³⁵ who said that RBO had better thermal stability than PO due to the volatile nature of carotene in PO during heating.

Oxidative stability of spreadable processed cheese analogues after three months of storage at 5 or 25°C:

The stability of processed cheese analogues as affected by replacing milk fat by edible oils (BO, RBO or PO) against hydrolysis was evaluated by the determination of both acid and TVFA values. Data presented in Table 5 showed that amounts of A.V for full-fat samples are significantly greater than half-fat and low-fat samples even in fresh or after storage for three months at 5 or 25°C. It can be also observed that there was a significant increase in A.V for all samples until the end of storage period. RBO containing samples had the highest A.V followed by BO and then PO and this trend was the same in all processed cheese analogue types and/or ages. The highest A.V was observed to be for full-fat processed cheese analogue containing RBO (0.78 mg KOH g⁻¹ oil) while the lowest was recorded for low-fat processed cheese analogue containing PO (0.39 mg KOH g⁻¹ oil). These results are in parallel with the A.V determined for raw materials (ROB, 1.02 mg KOH g⁻¹ oil; BO, mg KOH g⁻¹ oil and PO, mg KOH g⁻¹ oil) presented in Table 2. The TVFA values were also observed to be slightly significant increased for all samples until the end of storage. The greatest value was detected for full-fat BO containing samples (17.87 mL 0.1 N NaOH/100 g cheese) after three months of storage at 25°C. This attributes to the fat hydrolysis and liberation of free fatty acids, which cause gradual increase of rancidity by increasing storage temperature³³.

On the other hand, oxidation of fats or oils is considered as a complex process initiated by free radical reactions at the double bonds of unsaturated fatty acids. Therefore, the greater number of double bonds or degree of unsaturation of the fatty acids, the greater the susceptibility to oxidation. Some oils and fats are more prone to oxidation than others such as those high in unsaturated fatty acids, especially polyunsaturated fatty acids. Some of these oils and fats will have natural levels of antioxidants that can counteract the process of oxidation to a certain degree; however the protective effective will eventually be exhausted. This often coincides with an increase in oxidation of the unsaturated fatty acids and generation of rancidity. Under conditions of this study, the peroxide and TBA values were used to determine the oxidative stability of different produced processed cheese samples. Another index for oil oxidative stability is conjugated dienes. The first stages of fat oxidation can involve the formation of conjugated dienes and this can be measured spectrophotometrically at wave length 232 nm. Therefore, a change in conjugated dienes in fat containing products is considered as an index of oxidation as previously recommended by El-Shibiny *et al.*³⁶.

Table 5: Changes in the acid value (mg KOH g⁻¹ oil), peroxide value (meq O₂ kg⁻¹ oil), thiobarbituric acid (OD), dienes % and total volatile fatty acids (mL 0.1N NaOH/ 100 g cheese) of spreadable processed cheese analogues after three months under different storage temperature

Age	Type	Treatment	AV	TVFA	PV	Dienes	TBA
Fresh	Full	BO-F	0.40 ^{ij}	16.90 ^b	0.57 ^{hi}	0.34 ^{bc}	0.02 ^{bcde}
		RBO-F	0.61 ^{cd}	12.83 ^{ijk}	0.79 ^b	0.05 ^e	0.03 ^{ab}
		PO-F	0.32 ^{lmn}	13.83 ^{gh}	0.54 ^{ij}	0.03 ^e	0.03 ^{abcd}
	Half	BO-H	0.35 ^{klm}	14.90 ^{de}	0.46 ^j	0.32 ^{cd}	0.01 ^{de}
		RBO-H	0.55 ^e	10.77 ^{nopq}	0.63 ^{fg}	0.04 ^e	0.03 ^{abc}
		PO-H	0.29 ^{no}	11.23 ^{mno}	0.41 ^{mn}	0.03 ^e	0.02 ^{abcde}
	Low	BO-L	0.31 ^{mn}	11.97 ^{klm}	0.33 ^o	0.30 ^d	0.01 ^e
		RBO-L	0.46 ^{feh}	9.77 ^{rs}	0.45 ^j	0.03 ^e	0.02 ^{abcde}
		PO-L	0.26 ^o	9.43 ^s	0.33 ^o	0.03 ^e	0.02 ^{bcde}
3 months/5°C	Full	BO-F	0.48 ^f	17.33 ^{ab}	0.64 ^{efg}	0.36 ^{ab}	0.02 ^{cde}
		RBO-F	0.67 ^b	13.30 ^{ghij}	0.85 ^a	0.05 ^e	0.03 ^{abc}
		PO-F	0.40 ^{ij}	14.20 ^{efg}	0.61 ^{gh}	0.03 ^e	0.02 ^{abcd}
	Half	BO-H	0.42 ^{hi}	15.23 ^{cd}	0.52 ^j	0.32 ^{cd}	0.04 ^a
		RBO-H	0.60 ^d	11.10 ^{mnp}	0.71 ^{cd}	0.05 ^e	0.03 ^{abcd}
		PO-H	0.35 ^{klm}	11.63 ^{lmn}	0.47 ^{kl}	0.03 ^e	0.02 ^{bcde}
	Low	BO-L	0.36 ^{kl}	12.43 ^{kl}	0.40 ^{mn}	0.32 ^{cd}	0.01 ^e
		RBO-L	0.58 ^{de}	10.17 ^{qrs}	0.51 ^{jk}	0.04 ^e	0.02 ^{bcde}
		PO-L	0.33 ^{lmn}	9.93 ^{qrs}	0.38 ⁿ	0.03 ^e	0.02 ^{cde}
3 months/25°C	Full	BO-F	0.60 ^d	17.87 ^a	0.68 ^{de}	0.39 ^a	0.01 ^e
		RBO-F	0.78 ^a	13.43 ^{ghi}	0.88 ^a	0.05 ^e	0.02 ^{abcde}
		PO-F	0.47 ^{ie}	14.40 ^{def}	0.66 ^{ef}	0.04 ^e	0.02 ^{bcde}
	Half	BO-H	0.49 ^f	15.90 ^c	0.58 ^{hi}	0.34 ^{bc}	0.01 ^e
		RBO-H	0.68 ^b	11.33 ^{mno}	0.73 ^c	0.05 ^e	0.02 ^{bcde}
		PO-H	0.41 ^{hi}	11.90 ^{klm}	0.52 ^j	0.04 ^e	0.02 ^{cde}
	Low	BO-L	0.43 ^{ehi}	13.03 ^{hij}	0.44 ^{lm}	0.34 ^{bc}	0.01 ^e
		RBO-L	0.65 ^{bc}	10.40 ^{pqr}	0.54 ^{ij}	0.04 ^e	0.02 ^{cde}
		PO-L	0.39 ^{ijk}	10.20 ^{pqrs}	0.44 ^{lm}	0.04 ^e	0.01 ^{de}
LSD			0.047	0.934	0.044	0.031	0.016

BO-F: Full fat butter oil, BO-H: Half fat butter oil, BO-L: Low fat butter oil, RBO-F: Full rice bran oil, RBO-H: Half fat rice bran oil, RBO-L: Low fat rice bran oil, PO-F: Full fat palm oil, PO-H: Half fat palm oil, PO-L: Low fat palm oil, Different superscripts at the same column are significantly different ($p < 0.05$)

Results presented in Table 5 shows that peroxide values (P.V) of all types (full-fat, half-fat or low-fat) fresh processed cheese analogues containing RBO were higher than samples contained either BO or PO. The P.V increased significantly in different processed cheese samples by the end of storage period, but it was highest for full-fat sample containing RBO (0.88 meq O₂ kg⁻¹ oil) when stored at 25 °C. This may be due to the high level of polyunsaturated fatty acids in RBO which was 33.652% (Table 2) and can be easily oxidized³⁷. Samples containing PO presented lower P.V than those containing BO. This result was in agreement with the finding reported by Abd-El-Fattah³⁸ who found that soft cheese containing palm oil had lower PV compared with cheese samples containing butter oil. Regarding dienes and TBA as an oxidation indicator, data presented in Table 5 shows slightly changes in their values among all experimental samples when fresh or at the end of storage period.

CONCLUSION

From nutrition and health point of view it is well observed that substitutions of milk fat by edible oils are in increase. Under

current study the replacing of milk fat by butter oil, Rice bran oil or Palm oil in the spreadable processed cheese analogues were evaluated. Depending on the gained data we can conclude that the antioxidant activity and oxidative stability of cheese samples are highly affected by the type and percent of fat in the final product.

REFERENCES

1. Caric, M. and M. Kalab, 1993. Processed cheese products. Ch.15. In Cheese: Chemistry, Physics and Microbiology. 2nd Edn., Fox, P.F. (Ed.). Chapman and Hall: 2-6 Boundary Row: London SE1 8HN, UK, Vol. 2.
2. Calvo, M.V., M. Juariz, J. Fonteccha, M. El-Asar, M. Naguib and M.H. Abd El-Salam, 2007. Effect of milk fat replacement with vegetable oils on fatty acids composition and conjugated linoleic content of market Egyptian processed cheeses. Egypt. J. Dairy Sci., 35: 97-107.
3. Awad, R.A., W.M. Salama and A.M. Farahat, 2014. Effect of lupine as cheese substitution on the technological and nutritional properties of processed cheese analogue. Acta Sci. Pol. Technol. Aliment., 13: 55-64.

4. Cunha, C., R. Grimaldi, M.R. Alc ntra and W.H. Viotto, 2013. Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. *Int. J. Dairy Technol.*, 66: 54-62.
5. Solowiej, B., 2007. Effect on pH on rheological properties and meltability of processed cheese analogs with whey products. *Polish J. Food Nutr. Sci.*, 57: 125-128.
6. Stoon, A.E., 2002. The top 10 functional food trends. The next generation. *Food Technol.*, 56: 32-37.
7. Bachmann, H.P., 2001. Cheese analogues: A review. *Int. Dairy J.*, 11: 505-515.
8. Solowiej, B., I.W.Y. Cheung and E.C.Y. Li-Chan, 2014. Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins. *Int. Dairy J.*, 37: 87-94.
9. Lobato-Calleros, C. and E.J. Vernon-Carter, 1998. Microstructure and texture of cheese analogs containing different types of fat. *J. Texture Stud.*, 29: 569-586.
10. Muir, D.D., S.A.R. Williams, A.Y. Tamime and M.E. Shenana, 1997. Comparison of the sensory profiles of regular and reduced fat commercial processed cheese spreads. *Int. J. Food Sci. Tech. (Paris)*, 32: 279-287.
11. Gwartney, E.A., E.A. Foegeding and D.K. Larick, 2002. The texture of commercial full fat and reduced fat cheese. *J. Food Sci.*, 67: 2173-2183.
12. McMahon, D.J., M.C. Alleyene, R.L. Fife and C.J. Oberg, 1996. Use of fat replacers in Mozzarella cheese. *J. Dairy Sci.*, 79: 1911-1921.
13. Zhong, Q., 2003. Cooling effects on the functionality and microstructure of processed cheese. Ph.D Thesis. North Carolina State University, Raleigh.
14. Lee, S.K., R.J. Buwalda, S.R. Euston, E.A. Foegeding and A.B. McKenna, 2003. Changes in the rheology and microstructure of processed cheese during cooking. *Lebenson. Wiss. Technol.*, 36: 339-345.
15. Jones, S.A., 1996. Issues in Fat Replacement. in *Handbook of Fat Replacers*. Roller, S. and S.A. Jones (Eds.). CRC Press, Boca Raton, FL., pp: 3-26.
16. Drake, M.A., V.D. Truong and C.R. Daubert, 1999. Rheological and sensory properties of reduced fat processed cheeses containing lecithin. *J. Food Sci.*, 64: 744-747.
17. Abd El-Salam, B., 2015. Effect of milk fat replacement with vegetable oil and/or whey protein concentrate on microstructure, texture and sensory characteristics of fresh soft cheese. *Int. J. Dairy Sci.*, 10: 117-125.
18. Orthoefer, F.T., 2005. *Rice bran oil bailey's industrial oil and fat products*: John Wiley and Sons, Inc.
19. AHA., 2015. American Heart Association. *Fats and Oils: Fats 101. Scientific Report of the 2015 Dietary Guidelines Advisory Committee: Advisory Report to the Secretary of Health and Human Services and the Secretary of Agriculture.*
20. Kerckhoffs, D.A., F. Brouns, G. Hornstra and R.P. Mensink, 2002. Effects on the human serum lipoprotein profile of beta-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *J. Nutr.*, 132: 2494-2505.
21. Most, M., R. Tulley and S. Morales, 2005. Rice bran oil, not fiber, lowers cholesterol in humans. *Am. J. Clin. Nutr.*, 81: 64-68.
22. El Rhaman, S., 2010. Extraction and separation of γ -oryzanol from rice bran oil by HPLC and studying its effect on rats fed a high-cholesterol diet. *Int. J. Food Nutr. Public Health*, 3: 119-132.
23. Tuntragul, S., S. Surapat and P. Hongsprabhas, 2010. Influence of rice bran oil and rice flours on physicochemical properties of mozzarella cheese analog. *Kasetsart J. (Nat. Sci.)*, 44: 924-934.
24. A.O.A.C., 2000. Kjeldahl Method, Method No. 991.23. In: Horowitz, W., (Ed.). *Official Methods of Analysis of AOAC International*, 17th Edn., Gaithersburg, MD, USA., pp: 10-14.
25. A.O.A.C., 1990. *Official Methods of Analysis*. 15th Edn. Association of Official Analytical Chemists. Inc. Arlington Virginia USA.
26. Ling, E.R., 1963. *A text book of dairy chemistry*. Vol. 2. Practical, 3rd Edn. Chapman and Hall Ltd. London.
27. Mordi, J.C. and M.A. Akanji, 2012. Phytochemical Screening of the Dried Leaf Extract of *Cnidioscolus aconitifolius* and Associated Changes in Liver Enzymes Induced by its Administration in Wistar Rats. *Curr. Res. J. Biol. Sci.*, 4: 153-158.
28. Suwanna, V., M. Chittima, I. Lukkhana, S. Wikalda and K. Narisa, 2010. Examination of antioxidant activity and development of rice bran oil and gamma-oryzanol microemulsion. *J. Health Res.*, 24: 67-72.
29. Yildirim, A., A. Mavi and A.A. Kara, 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J. Agric. Food Chem.*, 49: 4083-4089.
30. Keeney, P.G., 1971. A guide to controlling oxidation in butter and creams. *Candy and Snack Industry*, 136: 68.
31. Kosikowski, F.V., 1986. *Cheese and Fermented Milk Foods*. 2nd Edn., Kosikowski, F.V. and Associates, Brooktonale, New York.
32. A.O.A.C., 1995. Ash of Cheese, Method No. 935.42. In: Horowitz, W. (Ed.). *Official Methods of Analysis of AOAC International*, 16th Edn., Gaithersburg, MD, USA., pp: 59.
33. Azaam, M.A., 2007. Effect of partial replacement of milk fat with vegetable oils on the quality of processed cheese spreads. *Egypt. J. Dairy Sci.*, 35: 87-95.
34. Abubakr, M.A.S., Z. Hassan, M.M.A. Imdakim and N.R.S.A. Sharifah, 2012. Antioxidant activity of Lactic Acid Bacteria (LAB) fermented skim milk as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Ferrous Chelating Activity (FCA). *Afr. J. Microbiol. Res.*, 6: 6358-6364.

35. Valantina, S.R., S.R. Shahayaraj and A.A. Prema, 2010. Antioxidant stability in palm and rice bran oil using simple parameters. *Rasayan J. Chem.*, 3: 44-50.
36. El-Shibiny, E. Safinaz, M.E. Shenana, Faiza M. Assem, G.F. El-Nagar and Sania M. Abdou, 2008. Oxidative stability of processed cheese spreads as affected by added whey proteins/carrageenan particulate. *J. Agric. Sci. Mansoura Univ.*, 33: 5177-5186.
37. Awad, R.A., S.A. El-Shabrawy and R.K. Singh, 2002. Texture and formulated emulsifying salt mixtures. *LWT-Food Sci. Technol.*, 35: 54-61.
38. Abd El-Fattah, A.M., 2006. Lipolysis and oxidative stability of fat in ultrafiltered white soft cheese containing vegetable oils. *J. Agric. Sci. Mansoura Univ.*, 31: 5193.